

RAPID COMMUNICATION

Plasma ghrelin concentrations in elderly subjects: comparison with anorexic and obese patients

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Abstract

Ghrelin, a novel endogenous ligand for the GH secretagogue receptor, has been reported to stimulate GH secretion and food intake in both humans and other animals. Interestingly, recent data indicate that ghrelin is up- and down-regulated in anorexia nervosa (AN) and obesity, which are also known to be accompanied by increased and reduced GH levels respectively. Ageing is associated with a gradual but progressive reduction in GH secretion, and by alterations in appetite and food intake. The role of ghrelin in the decline of somatotroph function and the anorexia of ageing is unknown.

To investigate the influence of age on circulating levels of ghrelin, a total of 19 young and old normal weight subjects (Y-NW, $n=12$; O-NW, $n=7$), six patients with active AN (A-AN), and seven patients with morbid obesity (OB) were studied. In addition to fasting plasma

ghrelin concentrations, baseline serum TSH, IGF-I and insulin levels were measured.

Mean plasma ghrelin concentrations in A-AN or OB were higher and lower respectively than those present in Y-NW. Interestingly, mean plasma ghrelin concentrations in O-NW were significantly lower than those present in Y-NW and superimposable on those of OB. The mean fasting plasma ghrelin concentrations in all groups of subjects were negatively correlated with body mass index and serum insulin levels, but not with TSH and IGF-I levels.

This study provides evidence of an age-related decline of plasma ghrelin concentrations, which might explain, at least partially, the somatotroph dysregulation and the anorexia of the elderly subject.

Journal of Endocrinology (2002) **175**, R1–R5

Introduction

Ghrelin, a 28 amino acid endocrine peptide expressed mainly in enteroendocrine cells of the stomach epithelium, was first characterized as the endogenous ligand for a new growth hormone secretagogue (GHS) receptor, which is present in various regions of the brain, including the pituitary, specific nuclei of the mediobasal hypothalamus and the hippocampus (Kojima *et al.* 1999, 2001).

In addition to acting as a potent growth hormone (GH) releaser, ghrelin also functions as a blood-borne orexigenic signal from the gut to the brain, in both humans and other animals (Wren *et al.* 2000, 2001). This effect is seemingly mediated, at least in part, via stimulation of a population of

arcuate nucleus neurons, which coexpress two orexigenic peptides – neuropeptide Y (NPY) and agouti-related protein (Kamegai *et al.* 2001, Wang *et al.* 2002).

Production of gastric ghrelin, which is released into the circulation, is regulated by nutritional and hormonal factors. Inhibitory signals seem to include leptin, insulin, GH and insulin-like growth factor-I (IGF-I), and a high-fat diet, whereas fasting and a low-protein diet have been related to increased plasma concentrations of ghrelin (Horvath *et al.* 2001, Toshinai *et al.* 2001, Lee *et al.* 2002). Interestingly, recent data indicate that ghrelin is down-regulated in human obesity (Tschop *et al.* 2001) which is also accompanied by reduced GH levels (Scacchi *et al.* 1999) and, conversely, plasma ghrelin levels are elevated in

Table 1 Demographic and hormonal characteristics of the study subjects. Values are means \pm S.E.M.; numbers are shown in parentheses

Group	Age (years)	BMI (kg/m ²)	TSH (mU/l)	Insulin (μ U/ml)	IGF-I (μ g/l)
Y-NW (12)	33.4 \pm 1.0	21.2 \pm 0.9	1.6 \pm 0.2	3.5 \pm 0.6	155.7 \pm 9.3
OB (7)	26.9 \pm 2.7	39.7 \pm 2.5 ^a	1.6 \pm 0.3	8.8 \pm 1.1 ^a	227.9 \pm 29.5 ^a
A-AN (6)	17.5 \pm 0.5 ^a	13.1 \pm 0.5 ^a	2.2 \pm 0.5	0.6 \pm 0.4 ^a	84.9 \pm 37.6 ^a
O-NW (7)	79.8 \pm 2.1 ^a	25.0 \pm 1.7	1.7 \pm 0.4	10.5 \pm 2.6 ^a	90.3 \pm 14.0 ^a

^a*P* < 0.01 vs Y-NW.

anorexia nervosa (AN), a GH hypersecretory state (Müller *et al.* 1995), and return to normal levels after partial weight recovery (Otto *et al.* 2001).

GH secretion declines with ageing in mammals, including humans, and it would seem that a defective pituitary function does not play a major role in this event (Müller *et al.* 1999). Rather, changes of somatotroph function that occur with ageing deal especially with the function of specific hypothalamic peptides for GH regulation, GH-releasing hormone (GHRH) and somatostatin (Müller *et al.* 1999). The discovery of ghrelin raises the issue of the physiologic role of the GHS/ghrelin system and forces profound revision of our current understanding of GH regulation in different GH-deficiency states, including ageing.

The aim of this study was to determine whether ageing modifies the plasma concentrations of ghrelin and to compare these levels with those present in physiopathological conditions where an alteration of GH secretion and nutritional status is present (i.e. obesity and AN).

Materials and Methods

Young and old healthy normal weight subjects (Y-NW, aged 27–39 years, *n* = 12; O-NW, aged 67–91 years, *n* = 7), patients with morbid obesity (OB, aged 16–36 years, *n* = 7) and patients with active AN (A-AN, aged 17–18 years, *n* = 6) were studied. Clinical (sex, age and body mass index (BMI)) and hormonal characteristics (insulin, IGF-I and thyrotrophin (TSH)) of the study subjects are shown in Table 1. All AN patients met the diagnostic criteria for AN according to the *Diagnostic and Statistical Manual of Mental Disorders IV-TR* (American Psychiatric Association 2000). Obesity was defined as BMI > 30 kg/m², according to the criteria of both the World Health Organization and the International Obesity Task Force (National Institutes of Health 1998). All obese participants were non-diabetic according to an oral glucose tolerance test, and healthy according to a physical examination and routine laboratory tests. Y-NW, O-NW and OB subjects had no history or actual evidence of endocrine or psychiatric disorders, and had not been taking

medications for the previous 6 months. Females with OB and Y-NW were all eumenorrhoeic and were studied in the early follicular phase of the menstrual cycle. Patients with A-AN had been amenorrhoeic for at least 3 months. All subjects gave informed consent for their participation in the study which had been approved by the Ethical Committee of our Institute.

Subjects were admitted to our Institute, where they received a weight-maintaining diet (50% carbohydrate, 30% fat and 20% protein) and abstained from exercise for at least 2 days before the study.

After an overnight fast, at 0800–0900 h blood was drawn into chilled tubes containing EDTA₂Na (1 mg/ml) and aprotinin (500 U/ml). Human plasma ghrelin was measured with a commercial radioimmunoassay (Phoenix Pharmaceuticals, Belmont, CA, USA) that uses ¹²⁵I-labelled bioactive ghrelin as a tracer molecule and a polyclonal antibody raised in rabbits against full-length octanoylated human ghrelin. No cross-reactivity was found with human secretin, human vasoactive intestinal peptide, human prolactin-releasing peptide-31, human galanin, human GHRH, neuropeptide Y or other relevant molecules. Sensitivity of the assay is 1 pg/ml. The inter-assay coefficient of variation (CV) was 9.0–13.6%, and the intra-assay CV was 4.5–5.3%. Serum IGF-I levels were estimated by chemiluminescence immunoassay (Nichols Advantage; Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Sensitivity of the assay is 6 μ g/l, and the inter- and intra-assay CV values are 6.3% and 4.2% respectively (1 μ g/ = 0.131 nmol/l). Serum insulin was measured by electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics GmbH, Mannheim, Germany). Sensitivity of the assay is 0.2 μ U/ml (= 1.39 pmol/l), and the inter- and intra-assay CV values are 8.2 and 4.2% respectively. In our laboratory, the normal range of serum insulin is 2.6–24.9 μ U/ml. Serum TSH was measured by ECLIA. Sensitivity of the assay is 0.005 mU/l, and the inter- and intra-assay CV values are 8.7 and 5.4% respectively. In our laboratory, the normal range of serum TSH is 0.27–4.2 mU/l.

Fasting plasma ghrelin concentrations were compared among the subgroups using analysis of variance. The relationships between fasting plasma ghrelin concen-

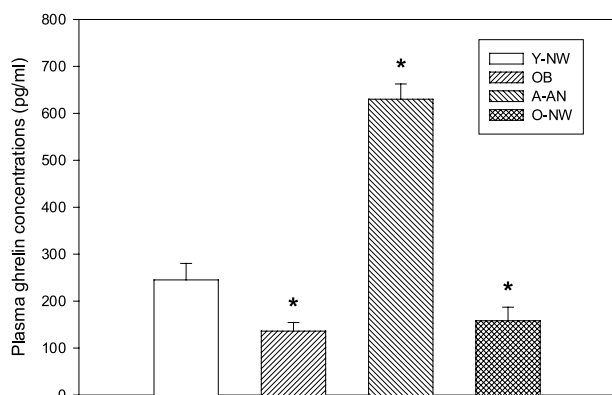


Figure 1 Fasting plasma ghrelin concentrations (means \pm S.E.M.; pg/ml) in Y-NW ($n=12$), O-NW ($n=7$), A-AN ($n=6$) and OB ($n=7$) subjects. * $P<0.05$ vs Y-NW.

trations and various anthropometric and metabolic variables (sex, age, BMI, insulin, IGF-I and TSH) were examined by linear regression and Spearman's correlation coefficient analyses. All of the analyses were performed using the SygmaStat version 2 software.

Results

Table 1 shows the mean values \pm S.E.M. for age, BMI, TSH, IGF-I and insulin in Y-NW, OB, A-AN and O-NW subjects.

Mean plasma ghrelin concentrations in A-AN or OB were higher (630 ± 32 pg/ml, $P<0.05$) and lower (136 ± 18 pg/ml, $P<0.05$) respectively than those present in Y-NW (245 ± 35 pg/ml) (Fig. 1). Interestingly, mean plasma ghrelin concentrations in O-NW (158 ± 29 pg/ml) were significantly lower than those present in Y-NW ($P<0.05$) and superimposable on those of OB (P =not significant) (Fig. 1). The mean fasting plasma ghrelin concentrations in these four groups of subjects were negatively correlated with BMI ($r^2 = -0.6$; $P<0.01$) and with serum insulin levels ($r^2 = -0.4$; $P<0.05$). There was no significant correlation between plasma ghrelin concentrations and serum IGF-I and TSH levels. In Y-NW, OB and O-NW subjects, no sex difference was observed in plasma ghrelin concentrations.

Serum insulin levels were significantly higher in OB and O-NW and lower in A-AN than those in Y-NW ($P<0.01$); serum IGF-I concentrations were significantly higher in OB and lower in A-AN and O-NW than those in Y-NW ($P<0.01$) (Table 1).

Discussion

Measurements of circulating concentrations of ghrelin in subjects with A-AN or OB were consistent with previous findings in the literature, when compared with values

present in Y-NW subjects, by showing enhanced titres in the former condition and, conversely, reduced values in the latter (Otto *et al.* 2001, Tschop *et al.* 2001).

AN is a psychopathologic disorder, which presents with neuroendocrine alterations reflecting, in a teleologic and perhaps reductive view, the needs of the organism to spare energy (Stoving *et al.* 1999a). Reportedly, GH secretion is increased in the florid phase of the disease, and, although this has been related to a loss of the feedback action of circulating IGF-I (Berelowitz *et al.* 1981, Counts *et al.* 1992, Ross & Chew 1995, Gianotti *et al.* 2000), a primary hyperfunction of GHRH-secreting neurons cannot be disregarded (Scacchi *et al.* 1997, Stoving *et al.* 1999a, 1999b, Gianotti *et al.* 2000, Pincelli *et al.* 2002). If this were the case, one cannot rule out the possibility that GHRH-secreting neurons are, in turn, under an enhanced stimulation by hypothalamic ghrelin-secreting neurons (Hewson & Dickson 2000). However, granted that such a mechanism is operative, it would not account for the almost 2.5-fold rise in circulating ghrelin concentrations that we have encountered in our study. It appears more likely that starvation and/or a low protein intake was stimulating secretion of gastric ghrelin in these subjects (Lee *et al.* 2002). Irrespective of the underlying mechanism(s), refusal of food in A-AN patients, who have high circulating concentrations of ghrelin and, presumably, high and low hypothalamic NPY and leptin functions respectively (Baranowska *et al.* 2001) bespeaks a down-regulation of the appetite-regulating system or a predominance of a central inhibitory system of energy homeostasis.

Previous measurements of plasma ghrelin concentrations in normal weight and obese Caucasian and Pima Indian subjects have disclosed the existence of a negative correlation between the above parameter and percentage body fat, fasting insulin and leptin concentrations (Tschop *et al.* 2001). In our study, fasting plasma ghrelin concentrations also correlated negatively in all subjects with BMI and fasting insulin. Many authors and ourselves believe that the decreased plasma ghrelin concentrations in obesity may represent a physiologic adaptation to the positive energy balance of this condition (Ravussin *et al.* 2001, Hansen *et al.* 2002).

Careful prospective clinical studies during weight loss or weight gain are mandatory to further clarify the role of ghrelin in the physiopathology of AN and obesity in humans (see also below).

The main finding of our study was the detection in O-NW of plasma ghrelin values as low as those present in OB and markedly lower than those of Y-NW, despite a BMI within normal limits and no overt signs of obesity, so that a deranged nutritional state cannot be invoked in these subjects. Increased insulin titres in O-NW would indicate an adaptive pancreatic mechanism to override the (age-related) insulin resistance (Garcia *et al.* 1997). Low serum IGF-I titres in O-NW, far from being a marker of malnutrition, reflect more likely the hyposomatotropism of

ageing (Italian Association for Research on Brain Aging 1997). Were a state of malnutrition present in the elderly subjects of our study, it would have been coupled to high, not low circulating levels of ghrelin. However, the lack of any correlation between circulating levels of ghrelin and IGF-I, as shown in this study and already reported by Dall *et al.* (2002), casts some doubt on the above interpretation (see also below).

Anorexia is a common event in ageing, and its occurrence involves both peripheral and central mechanisms (MacIntosh *et al.* 2000, Morley *et al.* 2001). It is tempting to speculate that low circulating levels of ghrelin, due to an impaired function of the gastric mucosa in the elderly, may contribute to the anorexia. The thickness of the gastric mucous membrane and the length of its glands, as well as the number of the endocrine cells, decrease in animals from puberty to old age (Khomeriki *et al.* 1986, Sandstrom *et al.* 1999). Also A-like cells, the main source of ghrelin in the stomach (Dornonville de la Cour *et al.* 2001), are likely involved in this age-related atrophic event. In this context, proof has been provided that ghrelin is a potentially important new peripheral signal to the brain to stimulate food intake in man (Wren *et al.* 2001).

Granted that a mechanism like this is operative in the elderly, a major issue is to clarify how the peripheral and the central components of ghrelin action are functionally interrelated in the O-NW, but also in A-AN and OB. To exemplify, GH function, which in the above conditions always parallels plasma ghrelin levels, reflects the action of the blood-borne peptide at a pituitary site, the involvement of ghrelin in the neuroendocrine control of hormone release or either mechanism. The finding that GH responses to ghrelin in male and female elderly subjects were lower than those of young subjects (Broglia *et al.* 2002) would be consonant with an hypothalamic-mediated effect.

However, the instances in which central and peripheral components of ghrelin action and GH plasma levels are divorced (Haqq *et al.* 2002, Katakami *et al.* 2002, Kletter *et al.* 2002) would imply a lack of relationship between the behavioural and neuroendocrine actions of the peptide.

In conclusion, the age-related decline of plasma ghrelin concentrations, evidenced by this study, might be related to the altered GH function and/or the anorexia of elderly subjects. However, before a definite conclusion can be drawn, a multivariate analysis on a huge number of patients is mandatory to definitively determine whether the decrease of ghrelin levels is due to ageing *per se*, or rather whether it is a merely a consequence of age-related changes in other parameters influencing synthesis/release of ghrelin, such as insulin and/or body composition.

Acknowledgements

This work was supported in part by MURST project 'Cofin 2000' MM05153399 to E E M.

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